Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (original) An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide having at least 80% sequence identity to a polypeptide encoded by a nucleic acid sequence selected from the group consisting of the *Pseudomonas aeruginosa* open reading frames (ORFs) listed in Table 1.

2. (currently amended) The isolated nucleic acid molecule of claim 1 wherein the sequence encodes a polypeptide having at least 90% sequence identity to said nucleic acid sequence.

3. (original) The isolated nucleic acid sequence of claim 1 wherein the sequence encodes a polypeptide having at least 95% sequence identity to said nucleic acid sequence.

4. (original) An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide having at least 80% sequence identity to a polypeptide encoded by an essential or important nucleic acid sequence selected from the group consisting of the *Pseudomonas aeruginosa* open reading frames (ORFs) listed in Table 1, wherein said essential or important nucleic acid sequence is identified as being essential or important by integration knock-out coupled with extra-chromosomal complementation.

5. (original) The isolated nucleic acid sequence of claim 4 wherein the sequence encodes a polypeptide having at least 90% sequence identity to said essential polypeptide.

6. (original) The isolated nucleic acid sequence of claim 5 wherein the sequence encodes a polypeptide having at least 95% sequence identity to said essential polypeptide.

7. (original) An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a polypeptide having at least 80% sequence identity to a polypeptide encoded by an essential or important nucleic acid sequence selected from the group consisting of the *Pseudomonas aeruginosa* open reading frames (ORFs) listed in Table 1, wherein said essential or important nucleic acid sequence is identified as being essential by integration of a regulatable promoter into the gene.

8. (original) The isolated nucleic acid sequence of claim 7 which encodes a polypeptide having at least 90% sequence identity to said polypeptide.

9. (original) The isolated nucleic acid sequence of claim 8 which encodes a polypeptide having at least 95% sequence identity to said polypeptide.

10. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the bacterial gene of claim 1.

11. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the bacterial gene of claim 2.

12. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the bacterial gene of claim 3.

13. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the bacterial gene of claim 1.

14. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the bacterial gene of claim 2.

15. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the bacterial gene of claim 3.

16. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 4.

17. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 5.

18. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 6.

19. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the essential or important bacterial gene of claim 4.

20. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the essential or important bacterial gene of claim 5.

21. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the essential or important bacterial gene of claim 6.

22. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 7.

23. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 8.

24. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 9.

25. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the protein encoded by the essential or important bacterial gene of claim 7.

26. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 8.

27. (original) A method of screening for an antibacterial agent, comprising determining whether a test compound is active against the essential or important bacterial gene of claim 9.

28. (original) The method of claim 13, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

29. (original) The method of claim 14, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

30. (original) The method of claim 15, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

31. (original) The method of claim 19, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

32. (original) The method of claim 20, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

33. (original) The method of claim 21, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

34. (original) The method of claim 25, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

35. (original) The method of claim 26, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

36. (original) The method of claim 27, comprising the steps of:

a) contacting said protein or a biologically active fragment thereof with a test compound; and

b) determining whether said test compound binds to said protein or said fragment; wherein binding of said test compound to said polypeptide or said fragment is indicative that said test compound is an antibacterial agent.

37. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 1, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

38. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 2, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

39. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 3, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

40. (original) A method for evaluating a test agent for inhibition of expression of the essential or important gene of claim 4, comprising:

a) contacting a cell expressing said essential or important gene with said agent; and

b) determining the amount or level of expression of said essential or important gene in said sample.

41. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 5, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

42. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 6, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

43. (original) A method for evaluating a test agent for inhibition of expression of the essential or important gene of claim 7, comprising:

a) contacting a cell expressing said essential or important gene with said agent; and

b) determining the amount or level of expression of said essential or important gene in said sample.

44. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 8, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

45. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 9, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

46. (original) The method of claim 37, wherein said level of expression is measured by measuring the amount of expression product in said cell relative to a cell that has not been contacted with said agent.

47. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 38, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

48. (original) A method for evaluating a test agent for inhibition of expression of the gene of claim 39, comprising:

a) contacting a cell expressing said gene with said agent; and

b) determining the amount or level of expression of said essential gene in said sample.

49. (original) The method of claim 37, wherein said level of expression is measured by measuring the level of expression of a gene fusion to said gene relative to a cell containing said gene fusion that has not been contacted with said agent.

50. (original) The method of claim 38, wherein said level of expression is measured by measuring the level of expression of a gene fusion to said gene relative to a cell containing said gene fusion that has not been contacted with said agent.

51. (original) The method of claim 39, wherein said level of expression is measured by measuring the level of expression of a gene fusion to said gene relative to a cell containing said gene fusion that has not been contacted with said agent.

52. (original) The method of claim 37, wherein said level of expression is measured by measuring the level of expression of a protein fusion to said gene relative to a cell containing said protein fusion that has not been contacted with said agent.

53. (original) The method of claim 38, wherein said level of expression is measured by measuring the level of expression of a gene fusion to said gene relative to a cell containing said gene fusion that has not been contacted with said agent.

54. (original) The method of claim 39, wherein said level of expression is measured by measuring the level of expression of a gene fusion to said gene relative to a cell containing said gene fusion that has not been contacted with said agent.

55. (currently amended) A method for evaluating ~~an~~ a potential antibacterial agent, comprising the steps of:

a) providing a bacterial strain comprising a mutant form of the gene of claim 1, wherein said mutant form of the gene confers a growth conditional or attenuated growth phenotype;

b) contacting bacteria of said bacterial strain with said test compound in semi-permissive or permissive growth conditions; and

c) determining whether the growth of said bacterial strain comprising said mutant form of a gene is reduced in the presence of said test compound to a greater extent than a comparison bacteria comprising a normal form of said gene.

56. (currently amended) A method for evaluating ~~an~~ a potential antibacterial agent, comprising the steps of:

a) providing a bacterial strain comprising a mutant form of the gene of claim 2, wherein said mutant form of the gene confers a growth conditional or attenuated growth phenotype;

b) contacting bacteria of said bacterial strain with said test compound in semi-permissive or permissive growth conditions; and

c) determining whether the growth of said bacterial strain comprising said mutant form of a gene is reduced in the presence of said test compound to a greater extent than a comparison bacteria comprising a normal form of said gene.

57. (currently amended) A method for evaluating ~~an~~ a potential antibacterial agent, comprising the steps of:

a) providing a bacterial strain comprising a mutant form of the gene of claim 3, wherein said mutant form of the gene confers a growth conditional or attenuated growth phenotype;

b) contacting bacteria of said bacterial strain with said test compound in semi-permissive or permissive growth conditions; and

c) determining whether the growth of said bacterial strain comprising said mutant form of a gene is reduced in the presence of said test compound to a greater extent than a comparison bacteria comprising a normal form of said gene.

58. (original) A library of nucleic acid sequences consisting essentially of nucleic acid sequences having at least 80% protein sequence identity to a nucleic acid sequence selected from the group consisting of the *Pseudomonas aeruginosa* open reading frames (ORFs) listed in Table 1, wherein said library of nucleic acid sequences is employed to identify essential genes in *Pseudomonas*.

59. (original) The library of 58 wherein said nucleic acid sequences encode proteins having at least 90% sequence identity to the open reading frames in Table 1.

60. (original) The library of 58 wherein said nucleic acid sequences encode proteins having at least 95% sequence identity to the open reading frames in Table 1.

61. (original) A map of at least about 10,000 to about 14,000 transposon insertions in the genome of *Pseudomonas aeruginosa,* wherein said map is useful for identifying genes that are essential or important for survival of said *Pseudomonas aeruginosa.*

62. (original) A vector comprising a promoter operably linked to the nucleic acid sequence of claim 1.

63. (original) A vector comprising a promoter operably linked to the nucleic acid sequence of claim 2.

64. (original) A vector comprising a promoter operably linked to the nucleic acid sequence of claim 3.

65. (original) The vector of claim 62, wherein said promoter is active in *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Hemophilus influenzae, Neisseria gonorrhea, Klebsiella pneumoniae,* and *Streptocooci.*

66. (original) The vector of claim 63, wherein said promoter is active in *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Hemophilus influenzae, Neisseria gonorrhea, Klebsiella pneumoniae,* and *Streptocooci.*

67. (original) The vector of claim 64, wherein said promoter is active in *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Hemophilus influenzae, Neisseria gonorrhea, Klebsiella pneumoniae,* and *Streptocooci.*

68. (original) A host cell comprising the vector of claim 65.

69. (original) A host cell comprising the vector of claim 66.

70. (original) A host cell comprising the vector of claim 67.

71. (original) A fragment of the nucleic acid of claim 1, 2 or 3 said fragment comprising at least 10, at least 20, at least 25, at least 30, or at least 50 consecutive bases of said nucleic acid.

72. (original) A protein having at least about 80% sequence identity to the protein encoded by the nucleic acid of claim 1, 2 or 3.

73. (original) A protein having at least about 80% sequence identity to the protein encoded by the nucleic acid of claim 4, 5 or 6.

74. (original) A protein having at least about 80% sequence identity to the protein encoded by the nucleic acid of claim 7, 8 or 9.

75. (original) An antibody or antibody fragment capable of specifically binding the protein of claim 72.

76. (original) An antibody or antibody fragment capable of specifically binding the protein of claim 73.

77. (original) An antibody or antibody fragment capable of specifically binding the protein of claim 74.

78. (original) An agent identified as having anti-bacterial activity by any of the methods of claims 10-57.

79. (original) A method for inhibiting the growth or survival of *Pseudomonas aeruginosa* comprising contacting said bacteria with an agent identified by a method as set forth in any one of claims 10-57 so as to inhibit growth or survival.

80. (original) A pharmaceutical composition comprising an agent according to claim 78.

81. (original) A method for treating a patient having a *Pseudomonas aeruginosa* infection, comprising administering to said patient an amount of an agent according to claim 78 effective to reduce or inhibit growth or survival of said *Pseudomonas aeruginosa*.

82. (original) A method of protecting a patient against a *Pseudomonas aeruginosa* infection, comprising administering to said patient an amount of an agent according to claim 78 effective to prevent said patient from acquiring a *Pseudomonas aeruginosa* infection.

83. (original) The isolated nucleic acid molecule of claim 4, 5 or 6, wherein said nucleic acid contains an essential gene selected from the group consisting of *Pseudomonas aeruginosa uppS, ispB* and *metK*.

84. (original) The isolated nucleic acid molecule of claim 4, 5 or 6, wherein said nucleic acid comprises the *Pseudomonas aeruginosa ispA* gene.

85. (original) The nucleic acid library of claim 58, 59 or 60, wherein said map is in electronic form.

86. (currently amended) The library of claim 85, wherein said electronic form is selected from the group consisting of magnetic storage media, ~~such as~~ a floppy disc, a hard disc storage medium, and a magnetic tape~~;~~, optical storage media, ~~such as~~ CD-ROM~~;~~, electrical storage media, ~~such as~~ RAM, ~~and~~ ROM~~;~~, hybrids of these categories, ~~such as~~ magnetic/optical storage media~~;~~, computer readable forms, ~~such as~~ a word processing text file, database format, searchable files, executable files and search program software.

87. (original) The transposon insertion map of claim 61, wherein said map is in electronic form.

88. (currently amended) The map of claim ~~85~~ 87, wherein said electronic form is selected from the group consisting of magnetic storage media, ~~such as~~ a floppy disc, a hard disc storage medium, and a magnetic tape~~;~~, optical storage media, ~~such as~~ CD-ROM~~;~~, electrical storage media, ~~such as~~ RAM, ~~and~~ ROM~~;~~, hybrids of these categories, ~~such as~~ magnetic/optical storage media~~;~~, computer readable forms, ~~such as~~ a word processing text file, database format, searchable files, executable files and search program software.

89. (currently amended) A method for identifying a library of putative essential or important genes using a High Throughput Transposon Insertion Database (HTTIM), comprising:

(a) mutagenizing a bacterial genome of a bacterium with a transposon such that individual cells containing at least one transposon insertion are isolated;

(b) collecting and mapping said at least one transposon insertion in each individual cell so as to form a database of transposon insertion sites, or an HTTIM;

(c) comparing said database of transposon insertion sites with a database comprising the genomic sequence of the bacterium to identify open reading frames in said genomic sequence database that are not disrupted by a transposon insertion;

(d) forming a library from said putative essential or important genes that are not disrupted by a transposon.

90. (currently amended) The method of claim 89, wherein said ~~bacteria~~ bacterium is *P. aeruginosa* or *S. aureus*.

91. (original) The method of claim 89, wherein said transposon inserts randomly into the target genome.

92. (original) The method of claim 91, wherein said transposon is Tn5.

93. (original) The method of claim 91, wherein said HTTIM comprises at least about 5000 transposon insertion sites.

94. (original) The method of claim 91, wherein said HTTIM comprises at least about 10000 transposon insertion sites.

95. (original) The method of claim 91, wherein said HTTIM comprises at least about 13000 transposon insertion sites.

96. (original) The library of putative essential or important genes identified by the method of claim 91, wherein said library comprises at most about 3000 genes.

97. (original) The library of putative essential or important genes identified by the method of claim 91, wherein said library comprises at most about 2500 genes.

98. (original) The library of putative essential or important genes identified by the method of claim 91, wherein said library comprises at most about 2000 genes.

99. (original) The library of putative essential or important genes identified by the method of claim 91, wherein said library comprises at most about 1700 genes.

100. (original) The method of claim 91, further comprising a statistical calculation for identifying putative essential or important genes.

101. (original) The method of claim 100, further comprising the statistical method applied herein.

102. (original) The method of claim 91, further comprising a physical mutagenesis experiment in order to verify essential or important genes.

103. (original) The method of claim 102, wherein said physical mutagenesis comprises knocking out a putative essential or important gene or creating a promoter swap mutant.

104. (original) An essential or important gene identified by the method of claim 102.

105. (original) An antibacterial agent that targets the gene of claim 104, or the gene product encoded by said gene.

106. (original) A pharmaceutical composition comprising said antibacterial agent of claim 105.

107. (original) A method of identifying a nucleic acid motif associated with a *Pseudomonas aeruginosa* infection, comprising screening the library of claim 58, 59 or 60 for conserved nucleic acid fragments.